REMARKS

Applicants wish to thank Examiner Schmidt for extending the courtesy of an interview on April 15, 2002, and for the helpful discussion that ensued.

Applicants have carefully considered the points raised in the Office Action and the interview and believe that the Examiner's concerns have been addressed as described herein, thereby placing this case into condition for allowance.

Status of the Claims

Claims 23-49 are pending in the application. Claims 1-22 were previously withdrawn from consideration as drawn to a non-elected invention. Claims 27, 29-31, 33, and 37 have been withdrawn as drawn to a non-elected species. Accordingly, claims 23-26, 28, 32, 34-36, and 38-49 are currently under consideration.

Applicants reiterate their request for inclusion of withdrawn species upon allowance of a generic claim, as permitted by 37 C.F.R. § 1.141(a).

The claim amendments are supported by the specification as follows:

Support for the amendments to claim 23 may be found for example on page 64, lines 15-26, page 65, lines 4-10, page 71, lines 3-24, and in Figure 26. Support for the amendment to claim 32 may be found for example on page 65, line 31 - page 66, line 6. Support for the amendment to claim 34 may be found for example on page 62, line 32 - page 63, line 2. Support for the amendment to claim 35 may be found for example on page 67, lines 5-15. Support for the amendment to claim 36 may be found for example on page 69, lines 12-16. Support for the amendment to claim 38 may be found for example on page 63, lines 17-23. Support for the amendment to claim 40 may be found for example on page 62, lines 1-2 and in Figure 26. Support for the amendment to claim 45 may be found for example on page 61, lines 26-28. Support for the amendment to claim 48 may be found for example on page 65, line 22. Accordingly, no new matter has been added by the foregoing amendments.

The title was amended to more accurately reflect the aspects of the invention that are currently under consideration.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**."

With respect to claim amendments, Applicants have not dedicated to the public or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants expressly reserve the right to pursue prosecution of any presently excluded subject matter or claim embodiments in one or more future continuation and/or divisional application(s).

Rejection under 35 U.S.C. § 112, first paragraph

Claims 23-26, 28, 32, 34-36 and 38-49 are rejected under 35 U.S.C. §112, first paragraph, as allegedly not enabled. Applicants respectfully traverse this rejection.

The Office Action reiterates arguments that the disclosure of Antisense-triplex-Ribozyme (ATR) molecules which have improved binding capacity over an Antisense-Triplex (AT) to the same target does not enable the claimed invention. However, the Office Action fails to address Applicants' previous argument that the claimed invention is enabled by the disclosure in Example VI of the specification.

Applicants respectfully reiterate that guidance regarding how to make and use the claimed invention is provided in Example VI of the specification (p. 56, line 29 - p. 71, line 24) and in Figures 25-29, 31, and 32. Example VI describes target-dependent RNA catalysis as a means for detection and amplification of target molecules.

Claim 23 as amended recites a method for detecting the presence of a target molecule in a composition, said method comprising contacting said composition with *a catalytically inactive RNA molecule which binds to said target molecule, wherein said RNA molecule comprises a catalytic domain*. The specification provides examples of RNA molecules which bind to the

target and comprise a catalytic domain. For example, the specification describes modification of a hairpin ribozyme to allow binding to a target sequence. As described on page 64, lines 16-19 of the specification and schematically in Figure 26, two RNAs comprise the hairpin ribozyme's catalytic domain E, a substrate binding sequence, and sequences complementary to adjacent regions of a target RNA in place of part of an essential helical stem of the catalytic domain. Figure 29 depicts nucleotide sequences for the two RNAs comprising the hairpin ribozyme's catalytic domain and target binding sequences. The nucleotide sequence information provided in Figure 29 is sufficient to enable one of skill in the art to make a catalytic ribozyme that binds to a target polynucleotide for use in the claimed methods. A further example is provided that involves binding of a hammerhead ribozyme to a target. Figures 31 and 32 depict the nucleotide sequences of the catalytic domain of a hammerhead ribozyme bound to a target. The binding site for the target molecule includes the ends of two of the three polynucleotide strands that comprise the hammerhead ribozyme (page 71, lines 19-23; Figures 31 and 32). The sequences provided in Figures 31 and 32 provide sufficient guidance to enable one of skill in the art to make a catalytic ribozyme for use in the claimed method. Thus, since the specification teaches nucleotide sequences for the catalytic domains of two different catalytic ribozymes, it would not require undue experimentation for a skilled artisan to make such ribozymes in accordance with the invention.

Claim 23 as amended further recites that *binding of said catalytically inactive RNA molecule to said target molecule allows catalytic action upon a substrate other than the target molecule.* The specification teaches how to produce a ribozyme that is catalytically inactive in the absence of the target and that will assume a catalytically active conformation only upon binding to the target, allowing catalytic action upon a substrate other than the target sequences to which it is bound. For example, the specification describes a catalytic domain of a hairpin ribozyme which contains sequences complementary to adjacent regions of the target in place of an essential helical stem (page 64, lines 16-19) Exemplary sequences for such a ribozyme are provided in Figure 29. The short stem in this ribozyme has been previously shown to render the ribozyme

unstable and catalytically inactive (page 64, lines 19-21). As disclosed in the specification, in the practice of the present invention, "the short stem is stabilized upon hybridizing of its ends to the target, thus creating the active conformation of domain E [of the hairpin ribozyme]" (page 64, lines 22-23). Creation of the active conformation upon binding to the target allows catalytic action upon a substrate other than the target (*e.g.*, cleavage of substrate sequences within a capture probe) (page 64, lines 23-26). The specification also provides methods for selecting a ribozyme with target-dependent catalytic activity. In particular, methods are disclosed for selecting a four nucleotide sequence (*i.e.*, the short stem sequence discussed above) from a randomized pool that will optimally allow target-dependent activation of an inactive ribozyme (page 66, lines 7-31). In a further example, the specification describes stabilization of a hammerhead ribozyme upon binding to a target, leading to cleavage of its substrate strand (page 71, lines 5-7). Nucleotide sequences for such a hammerhead ribozyme are provided in Figures 31 and 32. A target which is capable of stabilizing the catalytically active conformation of the ribozyme may be a polynucleotide (Figure 32), an aptamer (page 13, lines 4-7), or "any small molecule of interest, including proteins, small molecules, and metal ions" (page 71, lines 17-19). Thus, in view of the guidance in the specification and the specific teaching of nucleotide sequences for two different ribozymes which become catalytically active upon target binding towards a substrate other than the target, it would not require undue experimentation for a skilled artisan to make and use such ribozymes in accordance with the invention.

Claim 23 as amended further recites that *catalytic action upon the substrate is indicative of the presence of said target molecule in said composition*, and recites *detecting the presence of the target molecule, if any*. In methods of the invention, detection of catalytic activity of an RNA molecule indicates presence of the target molecule, since the RNA molecule is not catalytically active in the absence of target binding. The specification provides examples of methods for detection of catalytic activity of an RNA molecule that is catalytically activated upon binding to target. In one example, the specification describes the use of a hairpin ribozyme and a pair of replication probes which comprise the recognition sequence for Qβ replicase (page

65, lines 4-22; page 69, lines 12-16). When the hairpin ribozyme is bound to the target, it becomes catalytically active, allowing catalytic action (*i.e.*, ligation) upon the two replication probes. The ligated probes may then be amplified by Qβ replicase, indicating presence of the target molecule. An example of this procedure is depicted schematically in Figure 26. The specification teaches that amplified Qβ replicase products may be detected by, for example, addition of a fluorophore, such as ethidium or propidium iodide, and observation of increase in fluorescence as the reaction proceeds (page 68, lines 14-25). In another example, the specification describes the use of a hammerhead ribozyme (page 71, lines 5-24). The specification teaches tethering the substrate strand of the ribozyme to a solid support at one end and inclusion of a signal group, such as a fluorophore or biotin, at the other end (page 71, lines 7-10). Binding of the ribozyme to a target allows cleavage of the substrate strand of the ribozyme and consequently, separation of the signal group from the solid support (page 71, lines 5-12). Appearance of the signal group in solution may be quantitated by means that are well known to those of skill in the art, indicating presence of the target molecule. Alternatively, one end of the substrate strand may be attached to a fluorescent group and the other end to a quencher of fluorescence such that cleavage causes dequenching and an increase in fluorescence (page 71, lines 12-14). In view of the guidance in the specification, teaching methods for detection of catalysis by two different ribozymes upon target binding, it would not require undue experimentation for a skilled artisan to make and use such ribozymes in accordance with the invention.

In conclusion, the specification provides nucleotide sequences for two ribozymes which may be used in accordance with the methods of the invention and examples for selecting other ribozymes for optimal target-dependent catalysis. The specification also provides examples of methods for detecting presence of the target molecule. Thus, the specification provides sufficient guidance to enable one of skill in the art to make and use the invention without undue experimentation.

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

Rejection under 35 U.S.C. § 102(e)

Claims 23-26 and 28 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Bekkaoui et al. (U.S. Pat. No. 6,136,533). Applicants respectfully traverse this rejection.

In order to anticipate, a reference must disclose each and every element of a claimed invention. Bekkaoui et al. does not anticipate because it does not disclose the elements of a catalytic RNA molecule or target-dependent catalysis.

Bekkaoui et al. discloses a method for detecting a target nucleic acid molecule, comprising cleavage of a probe by RNaseH, a protein enzyme (see, *e.g.*, column 5, lines 31-33). All of the working examples provided in the specification involve use of RNaseH. There is no disclosure of any "enzyme" other than RNaseH and no disclosure or suggestion of use of a catalytic RNA molecule for catalysis. In contrast, in the presently claimed invention, catalysis is performed by an RNA molecule with a catalytic core. Further, in Bekkaoui et al., RNase H does not possess latent catalytic activity that is depend upon binding to a target. In contrast, the present claims recite a catalytically inactive RNA molecule which must bind to the target for catalytic action upon the substrate to occur.

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §102(e).

Rejection Under 35 U.S.C. § 102(b)

Claims 23-26, 32, 38-40 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Stefano (U.S. Pat. No. 5,472,840). Applicants respectfully traverse this rejection.

In order to anticipate, a reference must disclose each and every element of a claimed invention. Stefano does not anticipate because the disclosed methods do not include the claimed elements of binding of a catalytically inactive RNA molecule comprising a catalytic domain to a

target, or do not include catalysis dependent upon binding of the RNA molecule to the target. The methods disclosed in Stefano include either (1) an RNA molecule which contains target sequences as part of the catalytic domain (*e.g.*, Figs. 1 and 2), rather than an RNA molecule which comprises a catalytic domain in the absence of target sequences as presently claimed, or (2) capture of a catalytically active RNA molecule by the target, with activity dependent upon subsequent addition of a universal catalytic cofactor (Mg^{2+}), (*e.g.*, Figs. 3 and 4), rather than allosteric activation of a catalytically inactive RNA molecule by binding to the target as presently claimed.

One embodiment of the methods of Stefano is depicted in Figure 1. The specification describes this method as including a nucleic acid target which "has sequences which *participate in the formation of a ribozyme*" (column 17, lines 23-26, emphasis added). The remaining ribozyme sequences are provided by a separate nucleic acid which contains both target-binding and ribozyme sequences (column 17, lines 28-43). Similarly, Figure 2 depicts a hammerhead ribozyme in which a section of the target "is capable of *contributing sequences which form a ribozyme*." (column 18, lines 7-28, emphasis added). In contrast, the present claims recite contacting a composition with an RNA molecule which binds to a target molecule, wherein the RNA molecule comprises a catalytic domain. Thus, in the present invention, the RNA molecule includes a complete catalytic domain, and the target sequences do not contribute to or participate in formation of the catalytic domain.

Another embodiment of the methods of Stefano is depicted in Figures 3 and 4. In the methods shown in these figures, first and second nucleic acids create a hairpin ribozyme structure and are both bound to a target nucleic acid. Upon imposition of "ribozyme reaction conditions," the ribozyme causes cleavage at a cleavage site on the first nucleic acid, allowing this nucleic acid to become active and autocatalytically replicated upon imposition of autocatalytic replication reaction conditions. (column 18, line 43 - column 19, line 64) Thus, in this method, catalysis by the ribozyme (*i.e.*, cleavage at the cleavage site) is promoted by imposition of external "ribozyme reaction conditions." In contrast, the present claims recite that

binding of an RNA molecule to a target allows catalytic action upon the substrate. No other change of "ribozyme reaction conditions" is required. Further, in the method described in Stefano, the ribozyme is not dependent on target binding for catalytic activity. The target allows merely for capture or co-localization of the ribozyme. In contrast, in the present invention, binding to the target stabilizes the RNA structure in a catalytically active conformation. The RNA molecule in the present claims is catalytically inactive in the absence of target binding, and binding to the target is required for catalytic action upon the substrate to occur.

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §102(b).

Withdrawal of previous rejections

Applicants acknowledge with appreciation the withdrawal of the previous rejection under 35 U.S.C. §112, second paragraph, to the extent that it is not reiterated in this Office Action. Applicants would appreciate the Office officially withdrawing this rejection.

CONCLUSION

Applicants have, by way of the amendments and remarks presented herein, removed the issues for the rejections and addressed all issues that were raised in the outstanding Office Action. Accordingly, reconsideration and allowance of the pending claims are respectfully requested. If it is determined that a telephone conversation would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 367592000100. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the title

[Antisense and Antigene Therapeutics with Improved Binding Properties] Nucleic Acid Agents for Detecting Target Molecules and Methods for their Use

In the claims

23. (Twice Amended) A method for detecting the presence of a target molecule in a composition [suspected of containing said target molecule], said method comprising:

a) contacting said composition with a catalytically inactive RNA molecule which binds to said target molecule, wherein said RNA molecule comprises a catalytic domain,

wherein binding of said catalytically inactive RNA molecule to said target molecule allows [said catalytically inactive RNA molecule to become catalytically active towards] catalytic action upon a substrate other than the target molecule,

wherein [the action of the catalytically active RNA molecule on] said catalytic action upon the substrate is indicative of the presence of said target molecule in said composition; and

b) detecting the presence of the target molecule, if any.

32. (Once Amended) A method as in claim 23, wherein [the catalytically active RNA comprises] said catalytic domain is a catalytic domain of a hairpin ribozyme.

34. (Once Amended) A method as in claim 23, wherein [the catalytically active RNA catalyzes] said catalytic action comprises both cleavage and ligation of nonadjacent substrates that are both bound to the target.

35. (Once Amended) A method as in claim 23, wherein [the catalytic activity of the catalytically active RNA] said catalytic action comprises cleavage of a capture probe which is bound to the target and ligation of two replicase probes which are bound to the target.

36. (Once Amended) A method as in claim 23, wherein [the catalytic activity of the catalytically active RNA] said catalytic action comprises cleavage of a capture probe which is bound to the target[, said method further comprising] and ligation [by the catalytically active RNA] of two replicase probes which are not bound to the target.

38. (Once Amended) A method as in claim 23, wherein the substrate [is] comprises a capture probe which comprises [comprising a] polynucleotide sequences that are complementary to both the target sequence and [a] the substrate sequence [for the catalytically active RNA].

40. (Once Amended) A method as in claim 39, wherein said catalytic action comprises cleavage of the substrate and wherein a portion of the capture probe is released from the solid support upon said cleavage [of the substrate sequence by the catalytically active RNA].

45. (Once Amended) A method as in claim 44, wherein [the catalytically active RNA catalyzes] said catalytic action comprises ligation of the two replication probes to each other.

48. (Once Amended) A method as in claim 45, wherein [detection] detecting the presence of the target molecule comprises amplification of the ligated replication probes by Qβ replicase.